



- 2 -  
Serial Number 09/914,811

SEQUENCE LISTING

B/ <110> The University of Nottingham

<120> GENETIC SCREENING

<130> 16-007

<140> US 09/914,811

<141> 2001-08-30

<150> PCT/GB00/00761

<151> 2000-03-03

<160> 4

<170> PatentIn version 3.1

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<212> DNA

<213> Artificial

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<223> Description of Artificial Sequence: Primer. Part of vector sequence for the pZero-2 cloning vector (full sequence for the vector available from [http://www.invitrogen.com/vecseq\\_gcg/pzero2.seq](http://www.invitrogen.com/vecseq_gcg/pzero2.seq))

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22

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<212> DNA

<213> Artificial

<220>

<223> Description of Artificial Sequence: Primer. Part of vector sequence for the pZero-2 cloning vector (full sequence for the vector available from [http://www.invitrogen.com/vecseq\\_gcg/pzero2.seq](http://www.invitrogen.com/vecseq_gcg/pzero2.seq))

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<210> 3

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<212> DNA  
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B1  
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<212> DNA  
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<400> 4  
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Please replace the third full paragraph beginning on page 6 and extending to the top of page 7 with the following:

B2  
The initial mix of 43 probes was subcloned from plasmids and plasmid fragments known from sequencing or hybridisation not to contain dispersed repeat elements; in the case of one of the Y-linked probes (SRY), a 1360bp fragment was amplified using primers SRYA, SEQ ID NO: 3, (5'GCAGTAGAGCAGTCAGGGAG3') and SRYB, SEQ ID NO: 4, (5'GGGGAGAGAAAGAAACAAGTTTG3'). Other sources of cloned genomic DNA were: chromosome 1, pJBT2 (JALA, unpublished); chromosome 17, pYNZ22 (Nakamura et al., 1988); chromosome 18, pMS440 (Armour et al., 1990); chromosome 22, pMS632c (Armour et al., 1995); chromosome X, pMS613 (Armour et al., 1990). After isolation of the genomic insert and digestion with frequently-cutting restriction enzymes producing blunt ends (generally double digestion with *AluI* plus *HaeIII*), the resulting smaller fragments were cloned into the *EcoRV* site of pZero2 (InVitrogen) and propagated in *E.coli* TOP10 (InVitrogen). Care was taken to avoid repeat regions in DNA from minisatellite containing clones.

Please replace the first full paragraph on page 7 with the following:

B3  
With reference to Fig. 6, probes prepared by cloning blunt-ended restriction fragments from primary clones into the *EcoRV* site of pZero2 (InVitrogen) were amplified directly from bacterial cells (Sandhu et al., 1989) using flanking vector primers PZA, SEQ ID NO: 1, (5'AGTAACGGCCGCCAGTGTGCTG3') and PZB, SEQ ID NO: 2, (5'CGAGCGGCCGCCAGTGTGATG3'). The positions of these primers in the pZero-2 cloning site is shown in figure 6. PCR was carried out in Advanced Biotechnologies Buffer IV (75mM Tris-HCl pH8.8, 20mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% Tween), with 0.2mM each dNTP, 1mM MgCl<sub>2</sub>, 0.2μM each primer and 0.05U/μl Taq DNA polymerase (Advanced Biotechnologies). Reactions (generally 10μl) were subjected to 25 cycles of (95°C for 1 minute/70°C for 1 minute). Products amplified using <sup>33</sup>P end-labeled PZA were separated on denaturing 6% polyacrylamide/50% urea gels and probe mixes assembled such that the mobilities of all probes were distinct.